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Dopaminergic manipulations and its effects on neurogenesis and motor function in a transgenic mouse model of Huntington's disease

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ABSTRACT

Huntington's disease (HD) is an inherited neurodegenerative disorder that is classically defined by a triad of movement and cognitive and psychiatric abnormalities with a well-established pathology that affects the dopaminergic systems of the brain. This has classically been described in terms of an early loss of dopamine D2 receptors (D2R), although interestingly the treatments most effectively used to treat patients with HD block these same receptors. We therefore sought to examine the dopaminergic system in HD not only in terms of striatal function but also at extrastriatal sites especially the hippocampus, given that transgenic (Tg) mice also exhibit deficits in hippocampal-dependent cognitive tests and a reduction in adult hippocampal neurogenesis. We showed that there was an early reduction of D2R in both the striatum and dentate gyrus (DG) of the hippocampus in the R6/1 transgenic HD mouse ahead of any overt motor signs and before striatal neuronal loss. Despite downregulation of D2Rs in these sites, further reduction of the dopaminergic input to these sites by either medial forebrain bundle lesions or receptor blockade using sulpiride was able to improve both deficits in motor performance and adult hippocampal neurogenesis. In contrast, a reduction in dopaminergic innervation of the neurogenic niches resulted in impaired neurogenesis in healthy WT mice. This study therefore provides evidence that D2R blockade improves hippocampal and striatal deficits in HD mice although the underlying mechanism for this is unclear, and suggests that agents working within this network may have greater effects than previously thought.

Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder that is clinically defined by a movement disorder (typically chorea) together with cognitive and psychiatric disturbances. As in other neurodegenerative disorders, the functional abnormalities in HD have been proposed to result from a compromise of neural circuitry associated with cellular dysfunction or death (Ross and Tabrizi, 2011). The neuronal loss in HD is extensive, and in particular, many areas of the CNS including the hippocampus are affected by the disease process even in the earliest stages (Ross and Tabrizi, 2011). Indeed, HD transgenic (Tg) mouse models exhibit a range of hippocampal-dependent impairments, one of the best described being a reduction in adult neurogenesis in the dentate gyrus (DG) (Gil et al., 2005; Lazic et al., 2004; Phillips et al., 2005). During this process, endogenous neural precursor cells give rise to immature neurons which eventually mature and functionally integrate into the circuitry (Ming and Song, 2011), a process which is critical to some aspects of cognition (Clelland et al., 2009; Sahay et al., 2011).

Dopaminergic disturbances are also a common feature of the disease particularly a progressive reduction in striatal and extrastriatal D2 receptor (D2R) binding which begins before clinical manifestations and correlates with disease progression and frontostriatal cognitive impairment (Bäckman and Farde, 2001; Ginovart et al., 1997; Pavese et al., 2003, 2010; Ramos et al., 2013; van Oostrom et al., 2009). In line with human HD studies, there is evidence of an early reduction in striatal D2R expression in HD Tg mice (Benn et al., 2010; Cha et al., 1998; Cummings et al., 2006; Glass et al., 2004) although there have been limited approaches to demonstrate this in vivo using PET imaging as has been done in human HD patients. Furthermore, whether similar

Abbreviations: 6-OHDA, 6-hydroxyldopamine; D1R, dopamine D1 receptor; D2R, dopamine D2 receptor; DCX, doublecortin; DG, dentate gyrus; HD, Huntington's disease; L-DOPA, 3,4-dihydroxy-L-phenylalanine; micro-PET, micro-positron emission tomography; MFB, medial forebrain bundle; SVZ, subventricular zone; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

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changes occur in the dentate gyrus is unknown. Such questions have significant clinical interest as the majority of drugs used to effectively treat this disease block these receptors and/or deplete presynaptic dopamine (Mason and Barker, 2009; Priller et al., 2008).

We therefore sought to examine the dopaminergic system in HD not only in terms of striatal function but also at extrastriatal sites, especially the hippocampus. We used the R6/1 HD Tg mouse model which expresses exon 1 of the human huntingtin (htt) gene carrying 110–120 CAG repeats (Mangiarini et al., 1996). This line of HD mice has been well studied in terms of both the dopaminergic system and adult neurogenesis and also has a relatively slow evolution of progressive motor signs which makes them amenable especially to studies looking at the premanifest motor phase of the illness (Lazic et al., 2004; Ortiz et al., 2011; Petersen et al., 2002; Walker et al., 2011). We now demonstrate that in these HD Tg mice, using in vivo micro-PET imaging and mRNA expression, there is a significant reduction of D2R expression in the striatum and DG respectively which begins ahead of overt disease manifestations and neuronal loss in agreement with previous studies. Despite this reduction of D2R, further reduction of the dopaminergic input from a partial bilateral medial forebrain bundle (MFB) lesion paradoxically improved motor behavior and partially ameliorated adult hippocampal neurogenesis in the R6/1 mouse model of HD while having opposite effects in wild-type (WT) mice. Using the D2R antagonist which is commonly used to treat chorea and behavioral symptoms in HD, we observed a partial increase in hippocampal neurogenesis while there was no improvement in motor impairment. These findings have important clinical implications especially given that dopaminergic drugs are widely used in the treatment of patients with HD.

Materials and method

Animals

C57BL/6 wild type (WT) mice were purchased from Harlan Laboratories (UK). R6/1 Tg mice were purchased from the Jackson Laboratory (USA) and the colony was maintained by backcrossing to C57BL/6 females purchased from Harlan Laboratories (UK). After the mice were weaned, tissue from ear biopsies was sent to Largen Inc. (USA) for genotyping. All animals were kept in a temperature and humidity-controlled (22 °C) room on a 12-hour light/dark cycle. The mice were separately housed in single-sex cages of 3–4 mice per cage. Water and food was made freely available in the home cage. All experiments were performed using only female mice in the John van Geest Centre for Brain Repair, University of Cambridge, UK and Neuroscience Research Institute, Gachon University, Republic of Korea in strict accordance with appropriate Home Office project and personal licenses. Only female mice were used because of the need to avoid problems of fighting and sex hormone influences on any behavioral tests. The protocols were approved by the ethical committees of the University of Cambridge (UK) and the University of Gachon (Republic of Korea).

Micro-PET imaging

For the micro-PET study, animals were shipped via an air courier to NRI, Gachon University of Medicine & Science, Republic of Korea and allowed to recover for 2 weeks prior to scanning. R6/1 (n = 4) and WT littermate (n = 4) mice were scanned twice, at 12–13 weeks and at 21–23 weeks of age. Anesthesia was induced and maintained with passive oxygen/isoflurane at 1.5 l/min. Each mouse was positioned prone on a bed with its brain centered in the gantry and its head fixed by a nose bar. A PET scan was performed using a Focus 120 MicroPET system (Concorde Microsystems, Knoxville, TN) with 1.18 mm (radial), 1.13 mm (tangential) and 1.45 mm full width at half maximum (FWHM) resolution at the center (Kim et al., 2007). Dynamic scans were performed for 90 min immediately after a [C] raclopride injection (9.25–12.95 MBq, 200 μl) via the tail vein. The acquired data was reconstructed using a 2D-filtered back-projection algorithm (microPET ManagerTM, Siemens Medical Solutions, Knoxville, USA). Regions of interest (ROIs) for the striatum were drawn on the reconstructed PET images using a mouse brain atlas (Paxinos and Franklin, 2001). Regional radioactivity was determined for each dynamic frame in the ROIs. Tracer binding was calculated on the basis of the binding potential (BP) derived from a Logan plot graphical analysis technique (Logan et al., 1996).

mRNA expression

The DG was micro-dissected according to a previously described protocol (Hagihara et al., 2009) and samples were stored at −80 °C until use. RNA was extracted using an RNeasy Mini Kit (Qiagen, 74106) according to the manufacturer instructions and eluted in 30 μl of RNase-free water, the concentration of which was determined using a Nanodrop 2000c spectrophotometer (Thermo Scientific) and stored at −80 °C. cDNA was reverse transcribed using a Transcriptor First Strand cDNA Synthesis Kit (Roche) with anchored-oligo(dT) primers according to the manufacturer instructions. Quantitative Polymerase Chain Reactions (qPCR) were subsequently undertaken using Solaris Mouse qPCR Gene Expression Assays for D2DR. ATP5b was chosen as the reference gene due to its stable level of expression in HD mouse models when compared to other commonly used housekeeping genes (Benn et al., 2008). All qPCR reactions were performed in triplicates. Each dissection contained 3–5 R6/1 mice and 3–5 WT littermates and results were repeated with three independent dissections at different ages: 8, 12 and 16 weeks.

Dopamine lesion surgery

12–13 week old WT (n = 30 from Harlan Laboratories) mice or R6/1 (n = 22) mice and their WT littermates (n = 19) received bilateral injections of 6-OHDA (Sigma, UK) into the medial forebrain bundle (MFB). A stock of 6-OHDA was prepared in 0.01% ascorbic acid/saline and stored at −20 °C. The working doses were diluted daily and then carried on ice to the operating room. Mouse surgery was performed under isoflurane anesthesia using a mouse stereotaxic frame. The stereotaxic coordinates for MFB lesions were A/P: −1.1 (from bregma), M/L: ±1.1, and D/V: −4.7 (from the dura surface). Drugs were injected bilaterally at the rate of 0.5 μl/min through a Hamilton syringe (Hamilton Company, Switzerland) and the needle was left in situ for 5 min following delivery of the toxin to allow for its diffusion. For sham surgery, the identical volume of saline was injected using the same protocols as for the 6-OHDA injections.

Drug administration

Quinpirole and S-(−) sulpiride were purchased from Tocris (UK) and dissolved in saline with ethanol (quinpirole: to 100 mM and S-(−) sulpiride: to 10 mM, all of which were soluble in saline) using a warm bath (30 °C). Stocks of a constant volume were stored in a −20 °C freezer until use. 3,4-Dihydroxy-L-phenylalanine (L-DOPA, Sigma UK) was dissolved in saline using a warm bath on the day of administration (6 mg/kg) and was stabilized by co-injection with benserazide hydrochloride (12 mg/kg, Sigma UK). Stock drugs were dissolved in saline to obtain final working concentrations (quinpirole: 5 ml/kg, sulpiride: 10 ml/kg). Either drug or saline was injected intraperitoneally (i.p.) at 10 ml/kg volume in R6/1 (n = 27) and their WT littermate mice (n = 24).

Immunohistochemistry and cell quantification

Animals were perfused with 4% paraformaldehyde (PFA) and brains were post fixed overnight and then cryoprotected in 30% sucrose. Brains